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RESEARCH ARTICLE

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Cyclic thermal fluctuations can be burden or relief for an ectotherm depending on fluctuations' average and amplitude

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Abstract

1. Predicting the implications of ongoing ocean climate warming demands a better understanding of how short-term thermal variability impacts marine ectotherms, particularly at beyond-optimal average conditions during summer heatwaves.
2. Using a globally important model species, the blue mussel *Mytilus*, in a 5-week-long experiment, we (a) assessed growth performance traits under 12 scenarios, consisting of four thermal averages (18.5, 21, 23.5 and 26°C) imposed as constant or daily fluctuating regimes with amplitudes of 2 or 4°C. Additionally, we conducted a short-term assay using different mussel individuals to (b) test for the species capacity for suppression and recovery of metabolic performance traits (feeding and aerobic respiration) when exposed to a 1-day thermal fluctuation regime (16.8–30.5°C). Using this high-resolution data, we (c) generated short-term thermal metabolic performance curves to predict and explain growth responses observed in the long-term experiment.
3. We found that daily high-amplitude thermal cycles (4°C) improved mussel growth when fluctuations were imposed around an extreme average temperature of 26°C, representing end-of-century heatwaves. In contrast, thermal cycles negatively affected mussel growth at a less extreme average temperature of 23.5°C, resembling current peak summer temperature scenarios. These results suggest that fluctuations ameliorate heat stress impacts only at critically high average temperatures. The short-term assay demonstrated that during the warming phase, animals stopped feeding between 24 and 30°C while gradually suppressing respiration. In the subsequent cooling phase, feeding and respiration partially and fully recovered to pre-heating rates respectively. Furthermore, nonlinear averaging of short-term feeding responses (upscaling) well-predicted longer term growth responses to fluctuations.
4. Our findings suggest that fluctuations can be beneficial to or detrimental for the long-term performance of ectothermic animals, depending on the fluctuations' average and amplitude. Furthermore, the observed effects can be linked to fluctuation-mediated metabolic suppression and recovery. In a general framework,

we propose various hypothetical scenarios of fluctuation impacts on ectotherm performance considering inter- or intra-species variability in heat sensitivity. Our research highlights the need for studying metabolic performance in relation to cyclic abiotic fluctuations to advance the understanding of climate change impacts on aquatic systems.

KEYWORDS

acclimation, Baltic Sea, depression, elasticity, *Jensen's Inequality*, metabolic suppression, plasticity, stress

1 | INTRODUCTION

Thermal variations in marine ecosystems, particularly in coastal and shallow-water zones, can occur on short time-scales of minutes to days, due to changes in irradiance, up and downwelling and tides (Boyd et al., 2016; Choi et al., 2019). Ongoing climate change imposes decadal to centurial warming trends on marine environments (Rhein et al., 2013), affecting the characteristics of shorter term thermal fluctuations (Lima & Wethey, 2012; Sun et al., 2019; Wang & Dillon, 2014). For example, marine heatwaves are projected to become more frequent, prolonged and larger in amplitude (Hobday et al., 2016; Holbrook et al., 2019). Therefore, the probability of shallow-water ectotherms being exposed to beyond-optimal temperatures increases with ocean warming (Somero, 2010), and the severity of impacts will likely be influenced by the pattern of short-term (daily to week-long) fluctuations around these warming trends (Smale et al., 2019).

The performance of organisms in response to temperature is usually nonlinear, commonly represented by thermal performance curves (TPCs, Angilletta, 2006). The mathematics of nonlinear averaging (*Jensen's Inequality*; Jensen, 1906) predicts that, compared to a non-fluctuating thermal regime, the average response to a fluctuating regime with the same thermal average is higher for convex and lower for concave regions of an organism's TPC (Ruel & Ayres, 1999; Figure S1). Yet, such predictions assume that an organism's instant thermal response, as defined by its TPC, remains constant over time (i.e. lack of time-dependent effects; sensu Kingsolver et al., 2015; Sinclair et al., 2016). This assumption limits predictions on the consequences of thermal fluctuations for marine ectotherms with remarkable capacities for suppressing and recovering their (organism level) metabolic performance. This is particularly true for those organisms that evolved in variable environments such as shallow subtidal and intertidal habitats with pronounced diurnal or stochastic thermal fluctuations and recurring (tidal) aerial exposures (Helmuth et al., 2014).

In response to high critical temperatures, these ectotherms suppress their metabolic performance by temporally shutting down energy-demanding activities, such as feeding and growth, followed by reductions in aerobic respiration and, in some species, transition to anaerobic metabolism (Hui et al., 2020; Marshall et al., 2011; Sokolova & Pörtner, 2003). Metabolic suppression enables ectotherms to avoid excessive temperature-induced cellular demand for energy and metabolic substrates (Boutilier & St-Pierre, 2000; Pörtner, 2012; Ritchie, 2018). This, combined with heat shock protein upregulation, and ubiquitination and degradation of denatured proteins, can minimize heat-induced cellular damage (Han et al., 2017; Hofmann & Somero, 1995). Otherwise, heat-induced mismatch of metabolic supply and demand can lead to increased internal stress and energetic debt, ultimately leading to death when cellular ATPases cannot function at required rates to maintain ionic gradients (Boutilier & St-Pierre, 2000; Ritchie, 2018). A mismatch may also occur when metabolic suppression at constant but critical temperatures lasts for extended periods, negatively impacting performance over time (Schulte et al., 2011).

Therefore, TPCs of fitness proxies (growth, reproduction, development and survival rates) commonly established through days-to-months long exposures of organisms to static treatment conditions usually show concave drops at the higher beyond-optimal end (Deutsch et al., 2008; Martin & Huey, 2008). Thus, studies projecting the influence of thermal fluctuations on long-term performance using such TPCs may only yield negative impacts at high temperatures (Bernhardt et al., 2018; Paaijmans et al., 2013; Vasseur et al., 2014; Figure S1). Yet, according to some empirical findings, fluctuations can also positively affect the long-term performance of ectotherms (Bozinovic et al., 2011; Kang et al., 2019; Kingsolver et al., 2015; Niehaus et al., 2012). Hypothetically, alternations between phases of metabolic suppression that minimize stress, and phases of recovery, elicited by fluctuating regimes, might be responsible for these observations of fluctuation-enhanced performance (Schulte et al., 2011; Wahl et al., 2015). Refuge fluctuation effects on the long-term performance can be projected using metabolic TPCs generated on short time-scales of hours to days via an upscaling approach (Chesson et al., 2005; Denny, 2019; Denny & Benedetti-Cecchi, 2012). Such short-term TPCs usually show convex (decelerating) drops at the higher beyond-optimal end, manifesting heat-induced suppression of metabolic performance (Figure S1).

Through a long-term (5-week) experiment on a globally important model organism, the blue mussel *Mytilus*, this study first tests

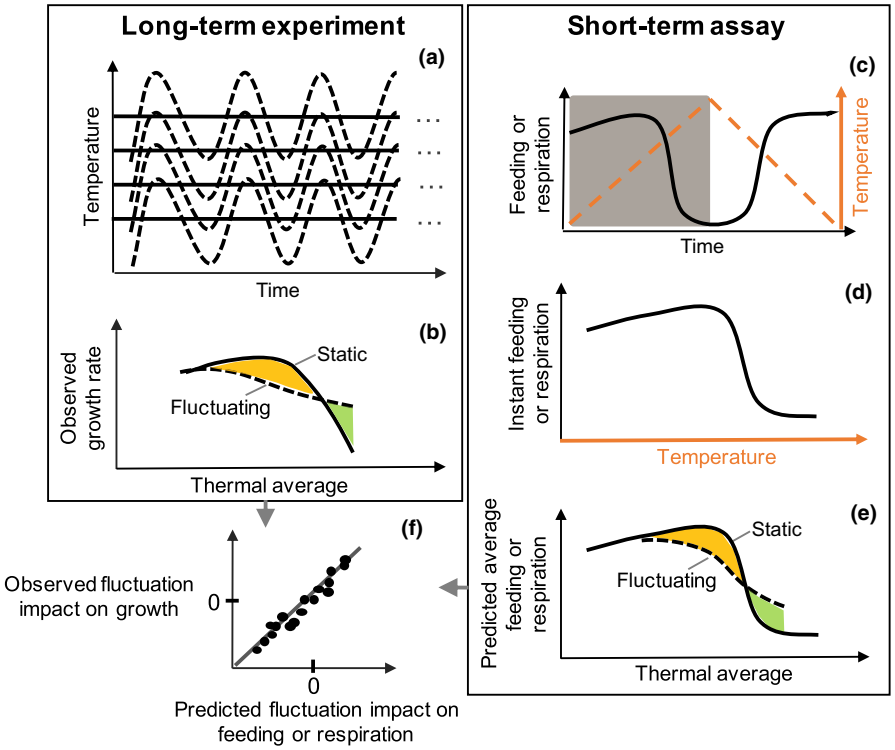

FIGURE 1 General sketch of the study workflow. Long-term experiment (a and b): (a) Mussel growth rates are evaluated in a 5-week experiment. Here, the exemplary treatments include four levels of thermal averages μ_T, potentially representing benign to high critical average conditions and two levels of fluctuations (continued and dashed lines for constant vs. fluctuating regimes). (b) Thermal performance curves (TPCs) describing the growth rate as a function of the thermal average are defined (solid and dashed lines represent growth under constant and fluctuating treatments respectively). The positive and negative effects of fluctuations around μ_T are shown by green and yellow areas. Short-term assay (c–e): (c) The organism-level metabolic performance (feeding and respiration rates) is recorded in response to a 1-day thermal cycle, documenting thermal metabolic suppression and recovery of the study organism. Using data from the warming phase of the cycle (denoted by the dark-shadow area), (d) the best-fit polynomial curve explaining the thermal metabolic response is selected, representing a short-term or non-acclimated metabolic TPC. (e) Upscaling from these short-term TPCs predicts the long-term average metabolic rates as a function of thermal averages and variability of the long-term experiment. Green and yellow areas indicate positive and negative fluctuation effects. (f) Finally, the relations between the fluctuations' long-term impacts on growth (observed) and metabolic performance traits (predicted) are assessed

the hypothesis that (a) daily thermal fluctuations can be beneficial to the growth of an ectotherm at high critical average temperatures. Furthermore, we conducted a short-term (1-day) fluctuation assay to test (b) the study species' capacity for suppression and recovery of metabolic performance (feeding and aerobic respiration) in response to the fluctuation with an amplitude representing the thermal range experienced in the long-term experiment. We finally (c) evaluated whether upscaling of the short-term thermal metabolic responses can predict the longer term impacts of fluctuations on growth observed in the 5-week experiment. A detailed workflow is given in Figure 1.

2 | MATERIALS AND METHODS

Our study organism is the marine filter feeder *Mytilus*, a foundation species complex dominating Baltic Sea mussel beds (Larsson et al., 2017; Stuckas et al., 2017). In the Western Baltic Sea (Kiel Fjord), *Mytilus edulis* is the dominant species, while the genome also includes small fractions of *M. galloprovincialis* and *M. trossulus* due to introgressive hybridization (Vendrami et al., 2020). The genus *Mytilus* has a world-wide distribution, and its various species and hybrids are known as ecosystem engineers creating mussel beds in the sub- and intertidal habitats of temperate- and cold-water ecosystems (Seed & Suchanek, 1992; Zippay & Helmuth, 2012). Notably, the Baltic Sea populations of *Mytilus* are usually submerged due to a minor (<10 cm) tidal shift of water levels in the region (Medvedev et al., 2016). Thus, the effects of temperature fluctuations on these mussels can be experimentally assessed without considering tidally induced aerial exposure as a covariate.

2.1 | Long-term (5-week) experiment

Four hundred *Mytilus* specimens with shell lengths of 2.5 ± 0.2 mm were collected in 0.5-m water depth from a hard-bottom area (50 m^2) in the Western Baltic Sea (Kiel Fjord), Kiel, Germany (54.4330891, 10.1711679) on 22 September 2018, at water temperatures of *c*. 16°C.

A subsample of 30 specimens was randomly selected and frozen to determine the mussels' initial size characteristics. From the remaining mussels, batches of 10 randomly selected individuals (hereafter, *group*) were placed inside a rigid mesh bag (1-mm^2 pore size, *c*. 10 cm^3 volume) distributed among 36 containers (2 L). Mussels were exposed to laboratory and container conditions (at 18.5°C) for 3 days. The containers were distributed among the 12 computer-controlled Kiel Indoor Benthocosms (Pansch & Hiebenthal, 2019). Mussels were exposed to the different average temperature treatments (18.5, 21.0, 23.5 and 26.0°C) by gradual (linear) warming of 2.5°C/day until the target temperature was reached. Over the next 5 weeks, mussels experienced 12 temperature scenarios, comprised of four thermal averages (18.5, 21.0, 23.5 and 26.0°C) imposed as constant or daily fluctuating regimes with amplitude of 2°C or 4°C.

Our nested experimental design is schematically described in Figure S2 in Supporting Information (Schielzeth & Nakagawa, 2013). Fluctuations were imposed as sinusoidal waves around constant averages to prevent possible confounding effects of unbalanced sequences of thermal exposures. Therefore, the treatments represent a simplified version of natural daily thermal cycles, which generally are characterized by higher stochasticity (Pansch & Hiebenthal, 2019). The logged experimental temperatures are plotted in Figure S3. The average temperatures applied in this study represent daily average temperatures for the maximum climatology (18.5°C; Pansch et al., 2018), current or near-future heatwaves (21 and 23.5°C) and a heatwave expected by the end of the 21st century during summer in the study region (26°C; see Gräwe et al., 2013). The daily fluctuation amplitudes (0, 2 and 4°C) used in this study represent conditions experienced by mussel populations at depths of 0.5–2.5 m in the non-tidal Western Baltic Sea, where the daily thermal change can be 3–6°C regularly and as high as 8°C occasionally during the warm season (Franz et al., 2019; Pansch & Hiebenthal, 2019). Notably, the treatment levels were likely to impose benign to critical temperatures since it was recently shown that the species could initiate suppression of metabolic performance at 23–25°C when exposed to a 24-hr fluctuation ranging from 18 to 27°C (Vajedsamiei, Melzner, Raatz, Kiko, et al., 2021).

During the experiment, mussels were fed a continuous flux of filtered (0.5 μm) seawater enriched with phytoplankton *Rhodomonas salina* at a flow of *c*. 3.5 ml/min from an independent source container (18 L). The positioning of the mesh bags and aeration mixing the food was kept equal between all containers and water baths. Nonetheless, mussel groups were redistributed between the three 2-L containers in each water bath every 3 days. The cryptophyte *R. salina* was cultured at 16°C and Kiel Fjord salinities by the Kiel Marine Organism Culture Centre at GEOMAR, KIMOCC. The food concentration in the source and experimental containers was measured every 5 days using a Cell and Particle Counter (Coulter Z2, Beckman Coulter GmbH) for the cell concentration (cells per ml; data are presented in Figure S4). The Coulter Counter was set to detect particles of 5–8 μm diameter, the typical *R. salina* dimensional range. Food concentrations allowing optimal filtration activity of *Mytilus* specimens (i.e. *c*. 1000–7000 *R. salina* cells/ml; Riisgård et al., 2006) were maintained throughout the assessments.

At the end of the experiment, study specimens were kept in 0.5 μm-filtered seawater at 18.5°C over 3 days to release remaining faeces, so faeces weight could not affect the mussel's dry tissue weight. Afterwards, the length of specimens was measured using a caliper, and their tissue was removed from the shell, both dried at 80°C for 24 hr and weighted using an electronic scale (±0.1 mg; Sartorius).

The response variables shell length (mm/day), mass growth and tissue dry weight growth (both mg/day) were calculated as fitness-related traits (Sebens et al., 2018). Each study specimen's final size was subtracted from the average initial size, and the difference was divided by the experimental duration. Averages and 95% confidence intervals of the responses to the different treatments were plotted group wise (Figure S5).

The significance of the main and the interactive effects for fixed factors (thermal average and fluctuation), and the effect of the random nested factor (i.e. group) were tested using Generalized Additive Mixed-effect Models (GAMM). The random (group) effects were negligible (for all three response variables, p-value >0.3). Thus, responses to each treatment combination were pooled over groups, meaning that feeding or respiration rates of 30 replicate mussels (except for dead mussels; see the Section 3) were grouped to define growth TPCs using fixed-effect GAMs (one TPC per fluctuation regime). The fixed-effect GAM performance was checked compared to more complex mixed-effect models based on AIC and adjusted R-squared and was found comparable. The average response was also compared between fluctuation levels (at each thermal average) using one-way ANOVA followed by post hoc Tukey HSD tests. Analyses were done using the packages MGCV and NMLE in R (R Core Team, 2019; see Script S1).

The simplest TPCs (fixed-effect GAMs) were fitted to data using the package PYGAM and plotted in combination with sample averages and 95% confidence intervals in Python (Python Software Foundation; see Scripts S2 and S3).

2.2 | Short-term (1-day) assay

Another set of *Mytilus* mussels was collected from a nearby shallow-water environment in Kiel Fjord (54.44655, 10.34551) on 20 November 2018, at water temperatures of *c*. 10°C, kept at constant 16°C for 3 weeks, and fed once per day with *R. salina* (KIMOCC) before the start of the assays. The short-term assay was composed of seven temporally repeated trials. During each trial, we recorded metabolic performance (feeding and aerobic respiration rates) of three different mussel specimens in response to a 1-day temperature fluctuation using our recently developed Fluorometer- and Oximeter-equipped Flow-through Setup (FOFS; Vajedsamiei, Melzner, Raatz, Kiko, et al., 2021). In the FOFS system, the phytoplankton food suspension was constantly pumped into four separate paths. Along each path, the suspension was first pumped into an air-tight incubation or oximetry chamber before entering a non-transparent fluorometry chamber. Using FOFS, we

recorded the mussel-induced reduction in food (via chlorophyll fluorescence) and dissolved oxygen concentrations as the difference between the records taken from three flow-through paths containing mussels and the records taken from the one mussel-free flow-through path. Continuous fluxes of a phytoplankton suspension with nearly constant algae concentrations into the incubation chambers maintained optimal food levels, thus enabling accurate determination of mussel routine metabolism. The initial data processing was done based on the protocol described in the study by Vajedsamiei, Melzner, Raatz, Kiko, et al. (2021). In short, we used robust regression techniques to remove the noise from the measured time series. The chlorophyll concentration measurement was time-lagged compared to the oxygen measurement because the chlorophyll sensor was positioned after the oximeter in each flow-through path of FOFS. The time lag was corrected through linear differential modelling. Finally, feeding and aerobic respiration rates were calculated based on the revised time series of measured variables.

In the short-term assay, mussels with a *c*. 20-mm shell length were used, allowing us to record individual-mussel responses using FOFS. In each trial, minimum and maximum temperatures experienced by the mussels were *c*. 16.8 and 30.5°C, respectively, covering the whole thermal range experienced by the specimens in the long-term thermal growth experiment. The rate of linear change over the warming and cooling phases was ±1.17°C/hour, and the times of minimum and maximum temperatures were reached at 5:00 and 17:00 respectively (Figure S6 temperature axes).

Data from 11 replicate mussels (Vajedsamiei, Melzner, Raatz, Sonia, et al., 2021) were processed as explained in the following. First, the time series of each response variable (see averages with 95% confidence intervals in Figure S6) were split into the warming and cooling phase series, based on the time intervals 5:30–16:30 and 17:30–4:30 respectively. Next, we grouped 11 replicated series of each phase and described the TPC of feeding or respiration with a polynomial model $g_p(T)$, where temperature T is the predictor variable, and a GAM in Python (see Script S4). The order of the best-fit polynomial model ($i \leq 10$) was selected based on the Bayesian information criterion (BIC), and the best-fit GAM was chosen from 700 models using a grid-search over many multiple regularization parameters and knots (4 to 10) seeking for the lowest Generalized Cross-Validation (GCV) score (Wood, 2017). The GAMs were only used to visually check the goodness-of-fit of polynomials since GAMs, in general, use the benefit of its spline basis expansion and the regularization (Wood, 2017).

2.3 | Predicting long-term metabolic rates by upscaling

To predict long-term-expected feeding or respiration rates $E(g)$ with the assumption of a lack of time-dependent effects (here, a lack of compensational acclimation or stress; Figure 1), we upscaled the polynomial TPC of the short-term assay's warming phase, which represented the non-acclimated thermal feeding and respiration responses. Upscaled thermal metabolic performance relations describing $E(g)$ as a function of thermal averages at fluctuating conditions were defined by taking the expectation of the ith-order Taylor expansion of the ith-order polynomial function around the predictor average (i.e. a type of *delta method for bias correction*; see Oehlert, 1992; Ver Hoef, 2012). The mathematical derivation can be found in Supporting Information Text S1. Upscaled relations were used to predict $E(g)$ as a function of thermal average and fluctuation scenarios of the long-term experiment. The procedure was done in Python (Script S4).

2.4 | Relating observed impacts on growth with upscaling-predicted impacts

The observed 5-week growth rate and the upscaling-predicted feeding and respiration rates were normalized using their minimum and maximum values (min-max scaled) under the constant treatments 18.5–26°C as 0 and 100 respectively (see Script S5). The relationship between the observed impact of fluctuations on growth and their upscaling-predicted impact on metabolic responses across thermal averages was tested with Pearson's correlation coefficient (see Script S5). The correlation analysis was only done for the high-amplitude fluctuation scenario (4°C) since it significantly impacted the mussels' growth traits.

3 | RESULTS

3.1 | Impacts of thermal averages and daily fluctuations on mussel growth during the long-term experiment

The GAMMs showed that, for all growth traits measured, the main effect of fluctuations was statistically not significant (p-values >0.05), while the main effect of thermal average and the interactive effect of thermal average and fluctuation were statistically significant (p-values <0.05; Table S1). Furthermore, ANOVA and subsequent Tukey HSD tests (Table S2) indicated that the average response to temperature was significantly different (p-values <0.05) between the two fluctuation amplitudes of 0 and 4°C, both at 23.5 and 26°C thermal averages for all growth traits and between 0, 2 and 4°C at 26°C for tissue growth only.

The resulting TPCs, describing rates of growth traits as functions of the thermal average, differed substantially between fluctuation regimes, particularly so at 4°C compared to treatments with 2°C fluctuation amplitude (Figure 2a–c; Table S1). The GAMs predicted decreases in growth traits for thermal averages between 20.5 and 25.5°C at the highest amplitude (4°C) compared to the static conditions (Figure 2a–c). In contrast, we found that growth increased in the fluctuating treatment compared to the static treatment at thermal averages beyond 25.5°C (Figure 2a–c).

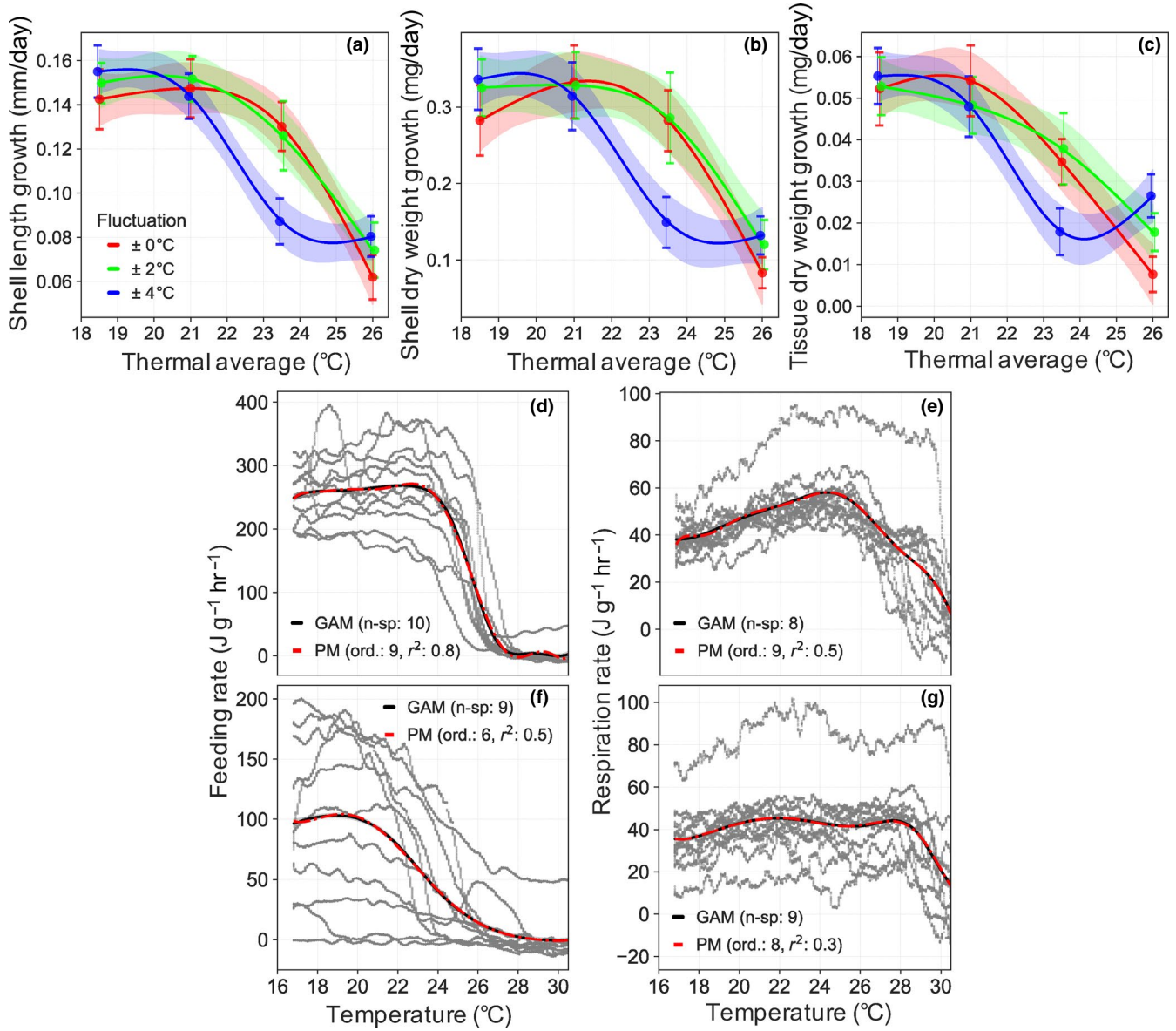

FIGURE 2 Thermal performance curves. (a–c) Thermal growth performance curves are retrieved from the long-term experiment. Generalized Additive Models (shaded areas represent 95% CIs) were fitted to data on variation in growth traits of mussel shell length (a) and shell and tissue dry weights (b and c) in 12 temperature scenarios (four average temperatures of 18.5, 21.0, 23.5 and 26.0°C with three diurnal fluctuation amplitudes of 0, 2 and 4°C). Sample averages with 95% CIs are shown as dots and whiskers. (d–g) Thermal metabolic performance curves as retrieved from the short-term assay. Thermal variation in rates of metabolic traits (feeding and respiration) during the warming (d and e) or cooling phase (f and g) of a diurnal fluctuation was described by the best-fit polynomial function (red dashed lines, order 6 to 9) and by Generalized Additive Models (GAMs; black lines; the number of splines 8 to 10). The best-fit GAMs were used to check the goodness-of-fit of polynomials visually. Grey lines indicate experimental data ($n = 11$)

3.2 | Metabolic performance during the short-term fluctuation assay

The best-fit polynomial functions describing the thermal metabolic responses of feeding and respiration over the warming and cooling phases of the 1-day-long thermal fluctuation of the short-term assay are presented in Figure 2d–g. In general, mussels suppressed respiration and feeding activity when exposed to high thermal extremes during the warming phase (Figure 2d,e) while recovering during the subsequent cooling phase (Figures S6; Figure 2f,g). The average feeding rate of mussels initially only slightly increased during the warming phase (Figure 2d). Beyond *c*. 23°C, a steep decrease in feeding rate could be observed, followed by a complete shutdown at 27°C. During the subsequent cooling phase, mussels gradually increased feeding rates, however only to a maximum level of *c*. 40% of the initial rate (Figure 2f). Respiration rate increased stronger during the warming interval and decreased from ca. 25°C onwards down to nearly 0 at *c*. 30°C (Figure 2e). However, respiration increased again during the subsequent cooling phase and recovered to the initial rate (Figure 2g).

3.3 | Upscaling-predicted impacts of fluctuations on feeding and respiration

The predicted rates of feeding and respiration for a hypothetical long-term fluctuation regime with the same characteristics as our long-term experiment are presented as min-max-scaled values in Figure 3a,b (also see Figure S7 for the responses in J g^{-1} hr^{-1}), together with the measured shell length growth patterns obtained from our long-term experiment (Figure 3c, see Figure 2a). In the treatments with daily fluctuations (±2 and 4°C), upscaling of the short-term performance predicts feeding and respiration rates to reach maximum values at lower average temperatures (Figure 3a,b) compared to the constant treatment, which is similar to the pattern observed for shell length growth (Figure 3c). At the average temperatures, 21, 23.5 and 26°C, long-term impacts of ±4°C fluctuations on feeding rates predicted from upscaling were comparable to the impact of fluctuations on long-term length growth observed in the long-term experiment (indicated by black arrows in Figure 3a,c). The decrease in feeding and growth at higher average temperatures is slower in the ±4°C fluctuation treatment compared to the constant (±0°C) treatment and finally results in relatively higher rates of length growth and feeding at thermal averages beyond 25.5°C. For the respiration rate, upscaling predicts decreasing effects of fluctuations for thermal averages of 21–26°C, with the maximum decreases at *c*. 24–26°C (Figure 3b).

3.4 | Correlating observed and predicted impacts of thermal fluctuations

We find that for average temperatures of 21–26°C, the impact of daily fluctuations (±4°C) on long-term-integrated growth rate is linearly correlated with the fluctuation impact on long-term-expected feeding rate predicted by upscaling. Pearson's correlation coefficients for shell length growth and growth of shell and tissue dry weights were 0.98, 0.98 and 0.81 respectively (Figure 3d; Figure S8).

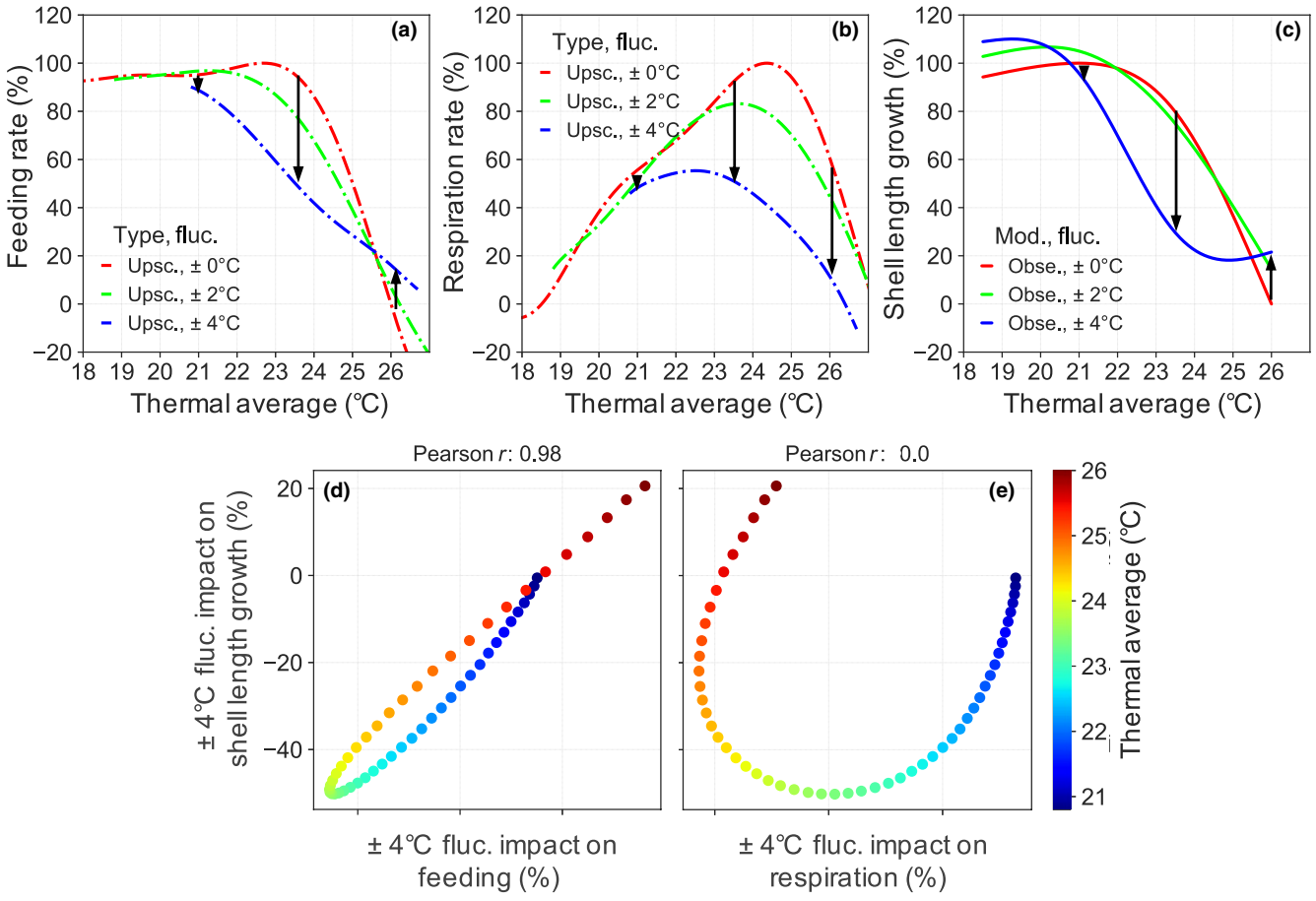

FIGURE 3 Effects of fluctuations on scaled metabolic rates (predicted) and growth traits (observed) at different thermal averages. (a, b) The upscaled thermal metabolic response relations obtained in the short-term assay were used to predict the long-term-expected rates of metabolic processes (feeding and respiration) at different average temperatures in response to the three scenarios of daily fluctuations of the long-term experiment. Predictions were min-max scaled, considering the minimum and maximum values of the constant treatment predictions. (c) Min-max-scaled shell length growth from the long-term experiment (see Figure 2a). Arrows indicate the consequences of large-amplitude fluctuations (±4°C) around the average temperatures of 21, 23.5 and 26°C. (d–e) The impact of large-amplitude fluctuations on growth observed in the long-term experiment is correlated against the upscaling-predicted impact of fluctuations on feeding and respiration rates obtained from the short-term assay

However, there were no or weak linear correlations for respiration rate (Pearson's correlation coefficients: 0, −0.03 and −0.46 respectively; Figure 3e; Figure S8).

4 | DISCUSSION

4.1 | Fluctuation benefits for long-term performance at critically high summer thermal averages

Using the blue mussel *Mytilus* as a model species, we provide supporting evidence to the hypothesis that short-term fluctuations can alleviate the longer term impacts of critically high average temperatures on an ectothermic organism. In our long-term experiment, we found that, compared to colder averages, mussel growth was substantially lowered by static exposure to 26°C, representing thermal averages of end-of-century marine heatwaves (Gräwe et al., 2013). Large-amplitude fluctuations, however, enabled mussels to improve their growth traits at an average temperature of 26°C, while the benefit of intermediate-amplitude fluctuations was minor. In contrast, mussel growth traits were only marginally affected by the static exposure to 23.5°C, representing conditions found during current or near-future marine heatwaves in the Western Baltic Sea (Holbrook et al., 2019; Pansch et al., 2018). At an average of 23.5°C, large-amplitude fluctuations substantially decreased mussel growth while intermediate-amplitude fluctuations had a minor effect. Therefore, in general, both the average and the amplitude of fluctuations were influential for long-term mussel growth, corroborating previous empirical findings for other ectotherms (Bozinovic et al., 2011; Cavieres et al., 2018; Niehaus et al., 2012; Siddiqui et al., 1973).

Shallow coastal waters of the Baltic Sea (depth *c*. 0.5–2.5 m) experience minimal tidal water-level changes. Nevertheless, daily variation in seawater temperature can be 3–6°C regularly and as high as 8°C occasionally during down- and upwelling events (Franz et al., 2019; Pansch & Hiebenthal, 2019). Even more intense fluctuations in body temperature can be observed at the low-latitude distribution range of *Mytilus* along the Atlantic coast, especially where specimens experience aerial exposure during low tides (Helmuth et al., 2014). Therefore, daily fluctuations are likely influencing mussel performance in these habitats and may particularly do so in a warming climate.

Some available literature reports detrimental effects of fluctuations for various species when comparing long-term performance at constant versus fluctuating thermal regimes (Bernhardt et al., 2018; Paaijmans et al., 2013; Vasseur et al., 2014). Yet, our findings suggest that fluctuations may be beneficial to the long-term performance of ectotherms at critically high average temperatures, corroborating some first empirical evidence (Bozinovic et al., 2011; Kang et al., 2019; Kingsolver et al., 2015; Niehaus et al., 2012). In the following, we discuss that these long-term impacts can result from fluctuation-mediated metabolic suppression and recovery (Schulte et al., 2011; Wahl et al., 2015).

4.2 | Metabolic suppression and recovery—potential benefits and costs during daily thermal cycles

Our studied mussels expressed the suppression and recovery of metabolic performance (feeding and aerobic respiration) in response to a 1-day thermal fluctuation between 16.8 and 30.5°C while being submerged. The mussels initiated feeding suppression followed by aerobic respiration suppression at about 23–25°C. During hours-long exposures to temperatures of 10 to 20°C, *Mytilus* respiration was shown to be more temperature dependent (Q_{10} of 2.1–2.5; Widdows, 1976) than filtration (Q_{10} of 1.25), the latter being driven mainly by the thermal change in viscosity of the surrounding solution (Kittner & Riisgård, 2005). A lower thermal dependence of filtration than respiration was also evident over the temperature range of 17–23°C applied during the warming phase of our short-term assay. This low thermal dependency of filtration might have partly helped mussels to control the ATP and oxygen demands of feeding and the associated energetic costs for digestion (i.e. typically *c*. 20% of the total mussel metabolic energy expenditure; Widdows & Hawkins, 1989) when the total metabolic energy demand was rising sharply due to increasing temperature. However, above a critical temperature threshold, feeding activities might have become too costly. Thus, filtration suppression poses a likely mechanism to decrease ATP and oxygen demand, enabling prolonged reserve use (Pörtner, 2012; Verberk et al., 2016).

Mytilus mussels closed their valves and shut down filtration and >90% of aerobic respiration during the warmest phase of the trial. To prolong survival time, hypoxia-tolerant (facultative anaerobe) species, such as *Mytilus*, can temporally shift to anaerobic metabolism and more efficient anaerobic pathways during phases of metabolic suppression. These yield less energy but prevent a thermodynamic collapse of cellular processes during severe heat stress events (Falfushynska et al., 2020; Gracey & Connor, 2016; Han et al., 2017; Podrabsky & Somero, 2004). Heat shock protein upregulation, and ubiquitination and degradation of denatured proteins might have also contributed to minimizing the heat-induced cellular damage (Han et al., 2017; Hofmann & Somero, 1995). The subsequent cooling phase of the cycle in the short-term assay likely provided an opportunity to rapidly recharge high-energy phosphate pools, reduce the concentration of accumulated anaerobic end products (e.g. succinate) and resynthesize storage compounds such as glycogen (*oxygen debt*, Ellington, 1983). In this recovery phase, mussels were characterized by high respiration rates in parallel to a partially recovered feeding activity. This reduction in ATP demand for feeding was likely to allocate the ATP (and oxygen) for the removal of the accumulated oxygen debt. This recovery process might have been impossible in a static thermal stress scenario where exhaustion of fermentable substrates, accumulation of energy debt, internal acidification and chronic membrane damage and leakage could occur and worsen the performance over time (Boutilier & St-Pierre, 2000).

Notably, evolution has resulted in various metabolic suppression pathways in ectothermic animals (Guppy & Withers, 1999). Compared to facultative anaerobes (such as *Mytilus*), obligate aerobic ectotherms

have a much lower capacity to suppress their ATP turnover rates and demand below baseline levels (Boutilier & St-Pierre, 2000). As a result, they have limited control over energy demand, mainly through the reduction of feeding, digestion and physical activity. However, when the heat-induced rise of demand exceeds their aerobic supply capacity, they can only compensate through time-limited reserve fermentation. Nonetheless, the capacity for recovery of both aerobes and facultative anaerobes during cool phases would depend on the magnitude of the energy and cell damage debt accumulated during the warm phases of thermal fluctuation regimes.

4.3 | Upscaling from short-term thermal feeding responses may predict long-term fluctuation impacts

Upscaling from the short-term thermal feeding relation using nonlinear averaging predicted the observed long-term impact of large-amplitude fluctuations on mussel growth well. This significant correlation suggests that fluctuation-mediated feeding suppression and recovery has contributed to decreased growth at less extreme average temperature (23.5°C) and to improved growth at a critical average temperature of 26°C. Importantly, nonlinear averaging using short-term feeding TPCs enabled us to predict refuge effects mediated by fluctuation regimes. As stated in the Introduction, such fluctuation-mediated refuge effects cannot be found in predictions using growth TPCs established by longer term static treatments (Bernhardt et al., 2018; Deutsch et al., 2008; Martin & Huey, 2008; Paaijmans et al., 2013; Vasseur et al., 2014). Notably, predictions based on nonlinear averaging of fluctuation impacts generally neglect time-dependent changes in TPCs. Therefore, such predictions can only be used as ecological null models (Dowd et al., 2015; Estay et al., 2014; Koussoroplis et al., 2019).

In contrast, upscaling from the respiration data obtained in the short-term thermal assay could not predict the long-term impacts of fluctuations on growth. Notably, respiration rate was predicted to be lower in the fluctuating compared to the static regime at the average condition of 26°C, while the observed growth rate was higher in the fluctuating regime. Considering a high energy cost of growth (i.e. *c*. 32% of the energy stored as new tissue in mussels or 34% of the total energy used by actively ingesting mussels; Clarke, 2019;Widdows & Hawkins, 1989), the higher growth should have been accompanied by a higher respiration rate to satisfy the ATP demand for growth processes (e.g. cell division costs, protein biosynthesis costs). This supports the general notion that short-term thermal respiration responses may not accurately represent long-term-expected respiration rates at beyond-optimal temperatures due to acclimation or stress effects (Semsar-Kazerouni & Verberk, 2018).

This study focused on testing whether nonlinear averaging using short-term (metabolic) TPCs can predict the long-term impact (benefits) of fluctuations, in terms of the average responses. Therefore, the inter-individual variances in mussel feeding and respiration responses to temperature (confidence intervals) were not upscaled. However, it is noteworthy to mention that inter-individual variability in heat stress sensitivity of metabolic traits might be selected upon during heatwaves and may contribute to adaptability of populations (Vajedsamiei, Wahl, et al., 2021).

Finally, the short-term assay results demonstrate uncoupling between mussels' short-term feeding and respiration TPCs when comparing the optimal thermal thresholds and relative temperature-induced changes in the responses, corroborating previous findings (Rall et al., 2012). This uncoupling suggests that the correction of temperature effects should be done independently for ingestion and maintenance processes in energy-budget modelling (e.g. DEB modelling; Kooijman, 2010) of ectotherms in thermally variable and beyond-optimal environments (Monaco & McQuaid, 2018).

4.4 | A framework indicating how thermal fluctuations may provide a refuge for ectotherms

In general, the characteristics of thermal fluctuations, such as the amplitude, period and time of occurrence (e.g. seasonality), as well as an ectotherm's functional traits, are essential factors defining how its metabolic performance may change during exposure to a constant or a fluctuating thermal regime (Bozinovic et al., 2013; Kingsolver et al., 2016; Semsar-Kazerouni & Verberk, 2018; Terblanche et al., 2007). This wide variety of influential factors may explain why empirical studies have sometimes obtained contrasting results regarding the long-term effects of thermal fluctuations at various thermal averages or in different ecological contexts (Bozinovic et al., 2011; Koussoroplis & Wacker, 2016; Niehaus et al., 2012; Siddiqui et al., 1973).

We propose a simple framework that may explain this context dependency based on possible scenarios of acclimation- or stress-induced changes in an ectotherm's capacity for thermal metabolic performance. In a simple model, such plasticity would manifest itself as a horizontal shift of the thermal metabolic performance curve (TPC), defining the instant performance response to temperature. When an individual is exposed to beyond-optimal conditions, whether constant or fluctuating, its capacity for temperature-dependent metabolic performance may remain constant or change due to acclimation or accumulation of stress (Fischer et al., 2010; Havird et al., 2020; Kingsolver et al., 2016; Precht, 1958; Terblanche et al., 2007). This translates into either an unchanged TPC, a right-shifted TPC or a left-shifted TPC respectively (Figure 4a–c; the darker grey shading denotes the critical temperature interval where metabolic performance is suppressed). Our framework acknowledges the general possibility that such shifts could occur independently for static beyond-optimal conditions and fluctuating beyond-optimal conditions with the same average. Upscaling these three TPCs for beyond-optimal constant conditions predicts three long-term performance expectations (black curves in Figure 4d–f). A similar upscaling procedure predicts the three different performance expectations for beyond-optimal fluctuating conditions (dashed blue curves in Figure 4d–f). Assuming their independence, this gives rise to nine possible combinations of long-term performance responses to

thermal averages under static versus fluctuating regimes (Figure 4g–o). The correlation between thermal fluctuation effects on long-term growth and feeding rates allows us to generalize these predictions to the long-term performance responses. It should be noted that we assume logit TPCs in this model by omitting the passive thermal dependence of metabolic performance (Schulte et al., 2011). For simplicity, the acclimation- or stress-induced changes in performance are considered only as changes in the curves' turning points and not their maximum or slope.

Beneficial effects of fluctuations are predicted in six out of nine hypothetical scenarios of the framework (Figure 4g,i,l,m–o), suggesting that the refuge effect of thermal fluctuations may indeed be a general pattern. A static exposure to extreme thermal conditions may stretch an organism's metabolic performance up to a level that initiates stress accumulation or prevents warm acclimation. Alternatively, the counterpart fluctuating regime with the same average as the static regime may provide a refuge if the duration and intensity of beyond-optimal exposures do not negatively impact the organism's capacity for elastic suppression and recovery of metabolic performance. In such conditions, thermal fluctuations may cause alternations between (a) phases of tolerance at high temperatures when the organism minimizes stress by matching the metabolic supply and demand at low levels, and (b) phases of recovery at lower temperatures when the organism enhances the performance to recover from metabolic debt, such as the oxygen debt and cellular heat damages experienced at high temperatures, and to refuel development, growth and reproduction.

Empirical evidence suggests that the capacity for compensational acclimation to extremely warm conditions is limited for organisms living close to their critical thermal thresholds, particularly those

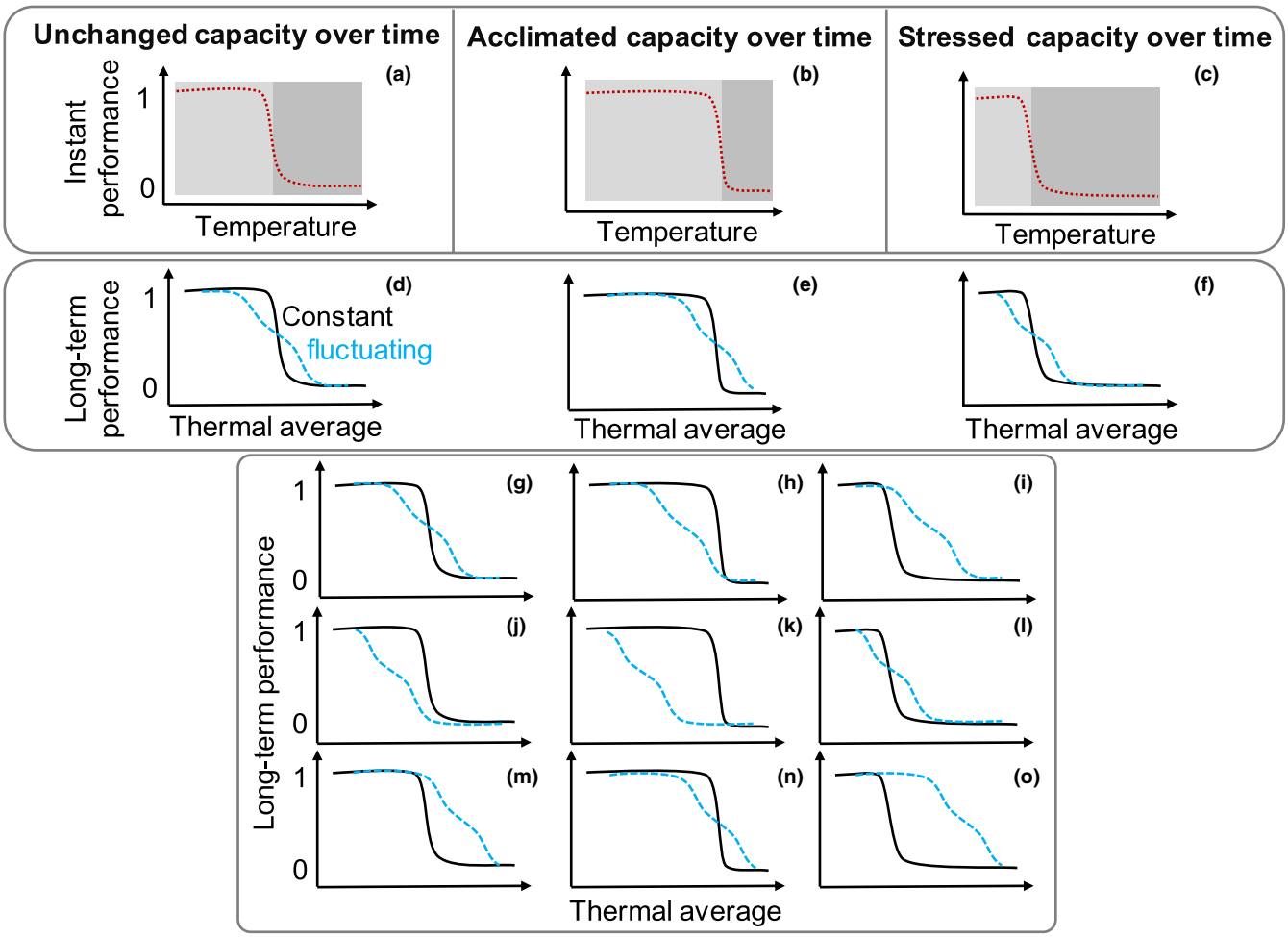

FIGURE 4 Mechanistic framework to understand the impact of fluctuations on ectotherms from highly fluctuating environments. (a–c, upper box) An organism's capacity for thermal metabolic performance (defining the instant performance) can remain constant or change over time by compensational acclimation or stress accumulation during exposure to beyond-optimal thermal conditions. These scenarios can be simply represented as an unchanged thermal metabolic performance curve (TPC), a right-shifted TPC or a left-shifted TPC respectively (dotted red curves). The grey shading separates the temperature interval of optimal (and near-optimal) metabolic performance from the critical interval where the performance is suppressed. (d–f, middle box) Based on the three possible TPCs, via nonlinear averaging, we can predict three general patterns of long-term-expected metabolic responses $E(g)$ to thermal averages under constant (black curves) or fluctuating regimes (dashed blue curves). (g–o, lower box) As acclimation to constant conditions may, in theory, be independent of acclimation to fluctuating conditions, we can predict nine hypothetical combinations explaining that thermal fluctuations may be detrimental for or beneficial to an ectotherm, depending on the context

adapted to highly fluctuating environments (Seebacher et al., 2015; Somero, 2010; Stillman, 2003), possibly due to a trade-off in favour of metabolic suppression capacity (McMahon et al., 1995). Our framework shows that even for organisms lacking strong warm acclimation capacity, fluctuations may still be beneficial (Figure 4g,i,l).

5 | CONCLUSION AND PERSPECTIVES

Our study suggests that cycles of metabolic suppression and recovery induced by daily thermal fluctuations can decrease or enhance the growth performance of blue mussels (*Mytilus*). The direction and strength of the effect strongly depend on the average and amplitude of the imposed fluctuation regime. This is an important finding, as with ongoing climate change, extreme temperatures and heatwaves will become more prevalent in coastal and shallow-water regions (Holbrook et al., 2019), likely increasing the relevance of this fluctuation-induced refuge effect.

The refuge effects mediated by thermal fluctuation regimes can be simplistically predicted through nonlinear averaging using TPCs of feeding performance generated at hour-long time-scales. On the other hand, such refuge effects typically cannot be predicted using TPCs for fitness proxies such as growth established in longer term but static exposures.

In this study, *Mytilus* was used as an ectotherm model species that has evolved in response to fluctuating stress regimes in coastal or shallow-water habitats where the capacity for metabolic suppression and recovery is an adaptive trait. Considering the variability in heat sensitivity of ectotherms and time-dependent effects (acclimation), we propose various hypothetical scenarios of fluctuation impacts. Testing the significance of these hypothetical scenarios for species expressing various suppression strategies can be a fruitful future research subject.

To mechanistically explain long-term fluctuation impacts on ectothermic species, it is essential to shift from studies applying static treatments to those that include natural system dynamics. More studies are needed to test whether and how acclimation and directional selection can induce changes in species capacity for thermal metabolic suppression and recovery (Norin & Metcalfe, 2019; Vajedsamiei, Wahl, et al., 2021) to better understand the ecological impacts of temperature fluctuations in a warming ocean.

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CONFLICT OF INTEREST

None declared.

AUTHORS' CONTRIBUTIONS

J.V. and C.P. designed the study; J.V. ran the experiments, analysed data and wrote the manuscript; F.M. and C.P. verified the experimental methods; M.R. verified the mathematical procedures; S.M. assisted J.V. during the long-term experiment. All co-authors discussed the results, reviewed and contributed to the final manuscript.

DATA AVAILABILITY STATEMENT

The data supporting the results are archived in PANGAEA repository https://doi.pangaea.de/10.1594/PANGAEA.933828 (Vajedsamiei, Melzner, Raatz, Sonia, et al., 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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